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Supplementary information

Meta-analytic evidence that animals rarely avoid inbreeding

In the format provided by the authors and unedited

Supplementary Methods

Study design

We searched the published literature for experimental studies in animals that estimated inbreeding avoidance at different episodes of sexual selection: before mating (i.e. 'precopulatory'), during mating, or after mating (i.e. 'postcopulatory'). We restricted our search to diploid animals where there was a comparison between kin and non-kin based on known familial relationships. Correlational studies (e.g. correlations between relatedness and level of extra-pair paternity) and studies that looked at same-sex associations were not included in our analysis. We considered individuals of different levels of relatedness (e.g. siblings, half-siblings, cousins) as 'kin'. In practice, for 'non-kin' the relatedness levels of the unrelated individual was generally assumed to be $r \sim 0$, although this is rarely quantified. Therefore, because we were not able to account for potential variance in relatedness level between the focal individual and the unrelated individual that was designated as non-kin, we proceeded with the assumption that non-kin levels of relatedness are $r \sim 0$. See decision tree in the Supp. Section 1 and https://osf.io/e34s9/ for full details on the inclusion and exclusion criteria.

Literature search

We performed a systematic literature search following guidelines from PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses[1]). The PRISMA diagram depicting our search and screening process is shown in Figure 5. We compiled a database of articles on inbreeding avoidance using the Scopus and Web of Science online databases in April and May 2019 accessed through the Stockholm University subscription. We updated our search in October 2019. We applied a broad scope to retrieve articles containing relevant data from various research areas. To this end, we used several search strings. Briefly, we started with a broad, general search to target any article containing 'inbreeding avoidance' (Search A). Second, we focused on synonyms of 'kin' in combination with

relevant parameters (e.g. mate choice, courtship, paternity; Search B). Third, we focused on 'kin recognition' studies that may still contain relevant data (Search C). Fourth, we performed a search focusing on inbreeding in combination with 'precopulatory' or 'postcopulatory' terms (Search D). Lastly, we focused on terms targeting human studies such as 'consanguineous', 'marriage', or 'incest' (Search E). The exact search strings used for each database are provided in https://osf.io/e34s9/. Based on the papers we were able to extract data from, we performed a backward search to find the studies cited in the 10 most recent papers in our searches (Backward search) and a forward search to find the studies that cited the 10 most cited papers in our searches (Forward search). Note that we conducted those searches based on Searches A-D, which focused on animals. We finally made a backward and forward search focusing on humans only by searching citations and cited papers on the papers we found relevant for humans in our original searches (Searches A-D) and the human specific search (Search E).

Eligibility criteria

The exact numbers of screened and included studies are shown in Figure 5, and the list of included studies in https://osf.io/e34s9/. We used Rayyan software to screen titles and abstracts[2]. We screened a total of 5619 unique abstracts. Two people (RdB and RV-T) screened the abstracts, using a decision tree (see Supp. Section 1). We had a partial overlap of decisions (600 abstracts were screened by both people, among which 37 had conflicting decisions). Conflicting decisions were discussed and resolved. Approximately 88% of the abstracts were removed because they did not fulfil our inclusion criteria, which left 688 papers for which we read the full text and applied the inclusion criteria. To be included for data extraction at the full-text screening phase, studies fulfilled the following criteria: (1) an experimental comparison between kin and non-kin via precopulatory mate choice, mating trials between kin and non-kin, or discrimination of kin via postcopulatory mechanisms in diploid animals. (2) Include a measure of inbreeding avoidance such as association or courtship with an individual (as a proxy for mate choice), mating success,

reproductive output, postmating mate guarding, remating rates, paternity bias, sperm investment, or sperm retention. We therefore excluded studies which only considered kin vs non-kin in agonistic (e.g. aggressive behaviours) or social (e.g. grooming, same sex behaviours) interactions not associated with mating behaviours. We also excluded egg viability, hatching success, or similar measures given these measurements are often confounded with inbreeding depression. (3) The study had to present comparisons of kin with non-kin, or present statistics that allowed for the comparison to be estimated. Thus, studies that only presented a correlation between relatedness and some measure of inbreeding avoidance were excluded. (4) Human studies where facial characteristics were computer-modified to resemble either the participant (self-resemblance) or another participant (unrelated) and measured the participant's attractiveness response. (5) The study had to provide sufficient information to estimate an effect size via descriptive or inferential statistics. Where sample sizes or variances were missing, we attempted to contact the authors for this information. All authors (n = 17) were asked if they could provide additional data that could be used in our analysis. Five authors replied to our data request and therefore included in our meta-analysis. A list of studies included and studies that did not fulfil our selection criteria are available from https://osf.io/e34s9/ along with the reason for their exclusion.

Data extraction and effect size calculation

We collected the means, standard deviations, and sample sizes of kin — non-kin comparisons. Data was extracted from text, tables, figures, or raw data. To extract data from figures we used the *metaDigitse* package (v.1.0[3]) in R (v. 3.6.0[4]). We transformed relevant study results into the standardised effect size Hedges' g[5]. Hedges' *g* expresses the difference in means in terms of standard deviations, and was used instead of Cohen's *d* because it is more robust to unequal sampling and small sample sizes[6]. We calculated the effect size in R using the *escalc* function in the *metaphor* package (v. 2.1-0[7]). Effect sizes

based on test statistics were calculated using the *compute.es* package[8]. See https://osf.io/e34s9/ for more details and an overview of the different effect size calculations.

We categorized avoidance mechanisms into (a) precopulatory (i.e. before mating), which we then grouped into association, attractiveness, and courtship, (b) during mating, which we grouped into mating (mating events or mating duration/latency) and reproduction (pairs of individuals producing offspring), and (c) postcopulatory (i.e. after mating), which included remating, guarding, paternity bias, sperm investment (number, velocity) for males, and sperm retention for females. We opted for this categorization because behaviours falling into 'precopulatory' could be regarded as measures of 'mate preference', whereas actual mating events could be regarded as measures of 'mate choice'. In addition, the behaviours included in the 'postcopulatory' category were often based on experiments with a focal animal, and thus a focal sex could be assigned, whereas behaviours included into the 'during mating' category were inherently the combined behaviour of two sexes. 'Postcopulatory' included behaviours that could only occur after a mating had already taken place. For those cases, we assigned a focal sex for sperm investment (=male effect) and sperm retention (=female effect). A mating status was assigned for males and females separately when the information was available. Avoidance mechanisms were always coded such that positive values represented inbreeding avoidance. Thus, effect size estimates obtained for relationships such as latency (e.g. to associate, or mate) were multiplied by -1 so that the positive estimates indicated that higher inbreeding avoidance was associated with higher expression of behaviours in which kin is avoided.

Moderator variables

In addition to the descriptive statistics for kin vs non-kin, we extracted the following information for each kin —non-kin comparison: taxonomic information (animal phylum, class, species), reproductive mode (egg-laying or livebearing), fertilisation mode (internal or external), relatedness, focal sex, episode of sexual selection (precopulatory, during mating,

postcopulatory), experience with kin (i.e. familiarity, where 'familiar' was defined by whether individuals spent time together during early development), sensory cues (visual, olfactory, auditory, tactile or a combination of these), choice type (whether animals were exposed to no-choice, sequential, simultaneous experimental choice type) and mating status (whether focal individuals were virgins or had mated before the test). See Supp. Section 2 for an overview of how these moderators were coded and see Box 1 for details on how each of these factors is hypothesized to influence inbreeding avoidance strategies in animals.

Statistical analyses

We performed our main analyses using the *rma.mv* function in the R package *metaphor*. We fit meta-analytic and multi-level meta-regression models. In all models, we used effect size identity (an observation-level unique identifier for each effect size calculated) and paper identity as random effects to account for nonindependence of the data from the same studies. Species and phylogeny were also included as random effects in all analyses to control for ecological differences and to account for the fact that more closely related species might show similar preference or aversion towards kin. The phylogeny used to compute a phylogenetic covariance matrix was constructed using the R Open Tree of Life package *rotl* (v. 3.0.10[9]), and branch lengths were set following Grafen's method (See Code available in https://osf.io/e34s9/). For all models we estimated heterogeneity using I^2 as an estimate of the proportion of variance explained in effect sizes due to differences between levels of a random effect following[10]. For models that included moderator variables we additionally report the marginal R^2 values (proportion of between-study variance explained by including the moderator) following[11]. We used the omnibus test of parameters ('Q_m'; provided by *metaphor*) as two-sided test statistic for the moderators. Point estimates were considered statistically significant when their confidence interval did not span zero.

We first calculated the overall mean effect size using meta-analytic models without considering any of the potential factors that can affect inbreeding avoidance (Box 1). We

constructed meta-analytical models with no fixed effects (i.e. intercept-only model), and included only random effects for between study variation, within study variation, species variation, and phylogenetic variation (see above). We did this for different subsets of the data based on confirmed important factors: the complete data set, only internal fertilizers, internal fertilizers and relatedness r = 0.5, and last internal fertilizers, relatedness r =0.5, and kin familiar / non-kin unfamiliar.

To examine the effect of factors that can affect the opportunity for animals to avoid inbreeding (Box 1), we then ran separate meta-regression models. For each model we included the same random effects as in the meta-analyses (between study variation, within study variation, species variation and their phylogenetic distance), but now also included each one of the moderator variables mentioned above.

Publication bias and sensitivity analyses

Publication bias was assessed based on funnel plots, with effect sizes (Hedges' g) plotted on the x-axis, and standard error on the y-axis. In the absence of publication bias the plot should be symmetrical, but asymmetry may be caused by other factors than publication bias, such as true heterogeneity or poor methodological design[12]. Distinguishing publication bias from other sources of asymmetry in the funnel plot was aided by graphically contouring areas of statistical significance using the *metaviz* package (v. 0. 3. 1[13]) . If asymmetry is caused by studies missing in areas of statistically insignificance, this would point to publication bias[14]. We additionally tested asymmetry in the funnel plot mathematically using Egger's regressions[15]. Egger's regressions were applied to the meta-analytic residuals of effect sizes (*sensu*[10]), calculated from the meta-analytical model using the *MCMCglmm* package (v. 2. 29[16]). The use of meta-analytical residuals fulfils the independence assumption, and they are less affected by heterogeneity[10]. We then used trim-and-fill tests to impute 'missing' (i.e. unpublished) studies from the literature based on funnel asymmetry[17] by using three estimators L_0, R_0 and Q_0 [18]. These estimators apply different algorithms that

can result in a different number of imputed “missing” studies as they focus on linear, rightmost run, and quadratic calculations, respectively[17-19], and we therefore applied all three estimators for comparison. We additionally tested for time-lag bias by including publication year as a single moderator variable in the model[20]. This was assessed in our meta-analytical models where data was subsetted. Additional sensitivity analyses were conducted to test whether results changed if Hedges’ g was calculated directly from continuous data or converted from an odds ratio based on proportional data, estimates were based on paired or independent data, and whether estimates shared a control group. In addition, we replaced the random effect for study identity with animal group identity (i.e. are the same animals used for all effect sizes from a single study or are there multiple groups of animals assessed within a single study) in our meta-analytical models to verify the study identity was still identifiable, which could occur if the number of species approaches the number of studies. None of these sensitivity analyses changed the results. See Supp. Section 10. All data and code are made available from https://osf.io/e34s9/.

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PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3-4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3-4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	23
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	23-24
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	23
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supp. material
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	23-24

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	24-25
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	25
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	26-27
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	24
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	25-26

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	26-27
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	27
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	5
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	Fig.3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Table 1
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Table 2, Fig. 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Table 3,

			Fig. 4, 10-17
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	19-23
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	17
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22-23
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	32

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

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